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Report No. IITRI-L6021-10
(Quarterly Progress Report)

DEVELOPMENT OF AN ORALLY EFFECTIVE INSECT REPELLENT

Headquarters
U.S. Army Medical Research
and Development Command
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IIT RESEARCH INSTITUTE

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I. INTRODUCTION

During this report period a literature survey was conducted in order to obtain a firm theoretical basis, in physiochemical terms, to better understand the binding of carbon dioxide by GABA. We have also somewhat modified the GABA hypothesis. This modification appears to render certain past experimental findings more comprehensible.

We continued testing compounds which are GABA* analogues for mosquito repellency, and continued with our development of a new instrument for obtaining repellency data utilizing the electronic recording method.

We also continued with the development of our statistical approaches, and made some modification in our computer program so that the data derived from our tests could be more efficiently handled.

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*Gamma amino butyric acid.

A number of compounds submitted by Dr. Ronald P. Quintana of the University of Tennessee were analyzed for repellency by our methods, as well as other compounds which are GABA analogues.

II. THE BINDING OF CARBON DIOXIDE BY AMINES, AND A MODIFICATION OF THE GABA HYPOTHESIS

An essential point in our hypothesis was that the binding of carbon dioxide (CO_2) by GABA must be easily reversible, and sensitive to temperature. We have shown in our past reports (1) that at constant temperature, the complex spontaneously dissociated, and that after 15 min only about one fifth of the carbamino compound remained compared to a solution which stood one minute before assay.

A number of Scandinavian workers who have been most recently active in the study of carbamino formation have also described the reversibility of these complexes with time and at constant temperature. The reversibility of a carbamino complex with amino acids at constant temperature was shown in detailed studies of Jensen and Faurholt (2). Olsen, et al (3) and Jensen et al (4) studied the carbamates of alkylamines, and Jensen et al (5) studied carbamate formation in amino alcohols. Reversibility was a characteristic of the reaction in all cases, and the complexes were practically entirely destroyed with time. In these cases, the carbamino compounds were gradually replaced by a stable equilibrium in which bicarbonate and carbonate ions were produced. Thus, the fact of spontaneous reversibility appears to be firmly established.

We next explored the question of the physical chemical aspects of the effect of temperature in the binding of CO_2 to amines. It is known that the only form of an amino acid which reacts with CO_2 is the anion, $H_2N\text{-}R\text{-}COO^-$, or more simply, R^- (ref. 6,7). (This represents a correction of a statement made in our last report, where the GABA molecule containing an uncharged carboxyl group was shown to participate in carbamino formation).

Thus: (a) $H_2N\text{-}R\text{-}COO^- + CO_2 \rightleftharpoons {}^-OOC\text{-}HN\text{-}R\text{-}COO^- + H^+$

or:

(b) $R^- + CO_2 \rightleftharpoons Am^{--} + H^+$, where Am^{--} represents the carbamino compound.

The equilibrium constant of the reaction is:

$$K_{Am'} = \frac{(Am^{--})(H^+)}{(R^-)(CO_2)}.$$

Pinsent, et al (ref. 8) studied the total heat evolved in the successive reactions:

(c) $NH_3 + CO_2 \rightarrow H_2H\text{-}COO^- + H^+$

(d) $H^+ + NH_3 \rightarrow NH_4^+$. ($\Delta H = -12.5$ kcal/M at 25°)

They found that ΔH = -9.0 kcal/M at 0°, -12.7 kcal/M at 20°, and -15.6 kcal/M at 40°. For reaction (d), ΔH at 25° is -12.5 kcal/M ($\Delta C_p = 0$) Substracting the ΔH of reaction (d) from that of reaction (c), we obtain a ΔH for reaction (b) of approximately +3.5 kcal/M at 0°, 0 kcal/M at 20°, and -3 kcal/M at 40° (ref. 9). The ΔH of formation of the carbamino compound is therefore very low, and extremely sensitive to temperature changes. On physical chemical grounds, the interactions of temperature with CO_2 and GABA that we have postulated are quite realistic.

The above reactions were performed in aqueous solutions. In the tissue, the H^+ released from GABA when complexing with CO_2 is probably taken up by tissue proteins, as well as other GABA molecules.

In terms of mosquito behavior, if the released H^+ combines with an amino group in the tissue to form $-NH_3^+$, then the above relationships probably hold, as for ammonium ion formation. If the temperature now increases as the mosquito approaches a host, the ΔH of the isolated carbamino reaction decreases. It reaches zero at about 20°, and then becomes slightly negative.

On the other hand, if the released H^+ combines with a $-COO^-$ group in the tissues, a relatively large positive entropy change occurs. In carboxylic acid ionizations, heat effects are negligible, and entropy changes constitute the most important contributions to free energy. Thus, both entropy changes as well as heat exchanges may occur. The entropy term is, therefore, probably also important in carbamino complex formation and destruction.

The ΔH of the separate reactants and products in the formation of carbamates appears to be quite low at ordinary temperatures, and extremely sensitive to temperature changes in this temperature region. The following table of constants for gamma-aminobutyric acid at 25° may also be of interest (ref. 10):

	pK_A	$\Delta F°$, cal/M	$\Delta H°$, cal/M	$\Delta S°$, cal/deg/M	ΔCp, cal/deg/M
GABA, pK_1	4.031	4400	405	-17.1	-34
GABA, pK_2	10.556	14400	12070	- 7.8	- 5

As can be seen, the ΔH of formation of GABA $(NH_3)^+$ is not very different from that in the case of ammonium ion $(NH_4)^+$, and similar thermodynamic relations probably exist. We could not find any references to the ΔH of formation of the carbamates of amino acids, and therefore cannot carry out the calculation for GABA. It appears from the above that although the net reaction is exothermic, the actual formation of the carbamate is endothermic at low temperatures, and exothermic at high temperatures. The result is that the ΔH of formation and decomposition of a GABA-CO_2 complex is such that the equilibrium is responsive to small temperature changes in the physiological range. We have shown experimentally that this is indeed the case (ref. 1). The GABA-carbamino complex is precipitously decomposed between 20° and 40°.

Pinsent (ref. 8) also determined the kinetics of reaction (c). He found the velocity constant, $k' = -d(CO_2)/dt \left[(CO_2)(NH_3)\right]$. to be 74 M^{-1} sec^{-1} at 0°, and 1130 M^{-1} sec^{-1} at 40°C. Thus

decomposition is very rapid as temperature increases. It is also of interest to note that CO_2 reacts with alpha alanine ($k' = 10^{4.82}$) approximately 1.5 times slower than with beta alanine ($k' = 10^{5.04}$) at 18° (ref. 2). This is not surprising on electrostatic grounds. A new negatively charged COO^- group is formed by the reaction of CO_2 with the amino group. Electrostatic work must be done against the repulsion of the negatively charged carboxyl group already present. These similarly charged groups are much closer together in alpha alanine than in beta alanine; therefore, the reaction would be expected to be slower with the alpha amino acid than with the beta form. It can be safely presumed on these grounds that CO_2 reacts very rapidly with GABA, since the distance between the carboxyl and the amino groups is comparatively great. Furthermore, it was shown by Stadie and O'Brien (ref. 11) that the velocity of carbamate formation depends upon the concentration of free CO_2. Thus, in a relatively high CO_2 environment, the GABA-CO_2 complex would probably form very rapidly.

It is probably correct to view the GABA in the insect as being present in more than one form. Part of it may be complexed with tissue, and there is evidence that in mammalian brain this indeed may be the case (ref. 12). It is also possible that the free GABA pool in the nervous structures of the mosquito waxes and wanes depending upon the nutritional state of the insect. In a condition of starvation, the free amino acid pool of the insect is depleted, and the GABA in the nervous structures would also probably be depleted. In such a condition, the insect would be much more sensitive to the presence of CO_2, and therefore to

the presence of a host. Less inhibitor is present initially, and therefore, less would have to be complexed with CO_2 to bring the insect to the activation threshold. The starved insect is thus more "avid" in terms of host-seeking behavior. Conversely, in a good nutritional state (i.e., after a blood meal), the GABA content in the nervous structures of the insect would be high. Since more GABA would have to react with CO_2, the insect would be more difficult to activate. This corresponds to what is known to be true of mosquito behavior in terms of responsiveness to CO_2 before and after a blood meal. Host-seeking behavior is also not usually immediately evident upon emergence from the pupa. This may also reflect the nutritional state of the insect, and therefore, of the free GABA pool.

Further considerations of the GABA hypothesis in the light of certain investigations by other workers have led us to believe that a modification of the hypothesis as originally stated may be in order.

It has been shown by various investigators (ref. 13-15) that neuromuscular preparations of crayfish respond to applications of glutamate with large depolarizations caused by activation of the excitatory synaptic membrane. Glutamate was also shown to have excitatory effects in neuromuscular preparations of insects. Kerkut et al (ref. 16) have shown that a profused cockroach leg preparation gave increased contraction after the addition of acetylcholine, L-glutamic acid, D-glutamic acid, and L-aspartic acid. It was further shown that GABA caused a marked inhibition

of the contraction, the inhibitory effect being quickly reverible with washing GABA had little inhibitory effect on contractions induced by acetylcholine, but could easily inhibit contractions caused by glutamic acid.

In a later paper by Kerkut and Walker (ref. 17), the effect of L-glutamate, acetylcholine, and GABA on the miniature end-plate potentials (mepps) and contractures of the coxal muscles of the cockroach were studied It was found that glutamate increased the amplitude and frequency of both mepps and contractures, and that GABA decreased these amplitudes and frequencies. The threshold concentration of L-glutamate for the increase was exactly equal to the threshold concentration of GABA for the decrease (10^{-6} g/ml) Thus, on a mole for mole basis, the mutually antagonistic effects of GABA and glutamic acid were nearly equivalent (the ratio is approximately 1 4 moles GABA to 1.0 moles of glutamate) In this preparation, acetylcholine had no effect on the mepps and contractures. Since glutamate was previously shown to be released during stimulation of the cockroach nerve in quantities related to the degree of stimulation (ref. 18) these authors suggested the possibility that glutamate is the excitatory transmitter in certain synaptic junctions of the cockroach.

The fact that GABA inhibited the mepps caused by glutamate and that acetylcholine had no effect on mepps in the coxal muscle in the cockroach preparation may indicate that a specific neuroinhibitory-neuroexcitatory system involving glutamate and GABA exists in certain insect synapses. In terms of our GABA

hypothesis, if one examines the structure of a GABA-CO_2 complex with the use of molecular models, it can be observed that the molecular conformation of the complex is not very different from that of glutamic acid. When we used N-acetyl GABA as an analogue of a GABA-CO_2 complex in the crayfish intestine preparation (ref. 19), we found that not only was there no inhibition, but the intestine was actually stimulated to contract. At first we could not understand these results, but now the situation appears more clear. The structure of N-acetyl GABA is also not very different from the structure of glutamic acid, and it is possible that the N-acetyl GABA mimics the effects of glutamate. A GABA-CO_2 complex is obviously a closer analogue of glutamate than N-acetyl GABA, and it is quite possible that the GABA-CO_2 complex is not only non-inhibitory, but actually stimulatory. Thus, the combining of CO_2 with GABA may not only remove a molecule of inhibitor, but add a molecule of stimulator. We have recently tested the effects of glutamate on our crayfish intestine preparation, and our preliminary experiments were in agreement with the results obtained by others (ref. 20). We found that glutamate does indeed stimulate contractions of the intestine of the crayfish.

This new view appears to lend even further support to the GABA-CO_2 hypothesis.

III. STATISTICAL ANALYSES OF REPELLENCY DATA AND MODIFICATION OF THE COMPUTER PROGRAM

The following is a summary of our approaches and objectives in the statistical analysis of repellency data.

The repellency of each compound is characterized by the percent of mosquitoes engorged out of approximately 50 that are exposed, and the percent displacement in the electronic recording during a 30 min test period (i.e., biting activity) compared to parallel control tests.

Each compound at each dose level tested yields a mean repellency index which is the sum of percent engorged and percent displacement, and the significance of differences of this index from the control values is tested taking into account day-to-day variation. A compound can then be judged by the maximum level of significance at which it tests significantly different from the control tests. A compound which has received more testing will be more likely to test significantly different from the control. This would also happen if the number of control tests is not the same for the days on which the compounds are tested. Day to day variation, which proved significant in every analysis of variance performed on control groups, is taken into account in such a way that days with lower average control values would be less likely to test significantly different if the test values are the same. In most cases, reduced biting for controls could be expected to be correlated with reduced biting for the test compounds. Such a response however, is impossible for test results which are zero or near zero. We know of no suitable

transformation to avoid this problem. The result is that the level of significance is of value primarily for testing the absolute difference from the control tests. Comparative ranking of test compounds can be made by testing these absolute differences at different concentrations of the compound, or by uniformly multiplying all control values by a desired fraction. The latter operation also provides proof of repellency by at least some percent below the control activity. The test of significance has therefore been adapted to test for significant deviation below any desired fraction of the control values. Thus we can state that our test compound has proved to be significantly repellent at 10% (or 50%) below the control values with some level of confidence. This operation was first introduced in the last report (ref. 19), and are also performed here (Appendix A).

We have recently reviewed the confidence levels which are stated for tests of significance of each compound. Since we were essentially interested in only the reduction of biting activity in testing our compounds, we had previously used a one-tailed test of significance (t-test). However, in view of the possibility of a slight skewing of the distribution of the repellency index, we have decided to take the slightly more conservative approach of using a 2-tailed test of significance. This change does not effect the program, but only the significance levels which are an input to the program. This will cause the significance levels in all cases to be shifted downward by one step Thus if in a previous test a compound was significantly

repellent at the 97.5% confidence level, it will now be significant at the 95% confidence level. Whereas we previously chose the 97.5% level as our accepted significance level, we will now accept the 95% confidence level as significant. All tests reported in this quarterly report will reflect this change.

Two major modifications have also been made in the computer program. In the first modification, the means for the controls for each day are computed automatically when the control data are input before the tests data, thus eliminating the need to separately calculate and input these means at the beginning of the program. The second modification eliminates the need for a separate analysis of variance (ANOVA) for each new group of tests. The variance for each new set of controls is now calculated, and an Analysis of Variance table is printed by the computer. The ANOVA appears immediately after the controls, with an F-test for significance of the day effect (The F-value in this data shows that the day effect is significant). The computations for the sum of squares (SS), degrees of freedom (DF), and mean square (MS) are also printed. The error mean square (error MS) is the control group variance which is actually used in the subsequent tests of significance. This value is 745.569 in the present analysis.

During this report period we performed another Discriminant Function Analysis to retest the validity of our repellency index. We previously found using this type of analysis (ref. 21) that the sum of the percent of total number of exposed mosquitoes which engorged plus the percent of total time displacement from the base

line in the electronic recording method was sufficient to adequately separate controls from the low level treatment (borderline) cases. The results of our recent re-analysis using new data showed that this method of distinguishing control from test groups is still valid, and there was not shown to be a statistical basis for changing our approach. The efficiency of the repellency index will continue to be reevaluated from time to time.

During this report period we also tested an innovation of our repellency testing method. We "conditioned" our mosquitoes by placing the wire mesh on the top of the container 24 hr before the group of mosquitoes were actually used for the test. Previously, the mesh was placed on top of the mosquito container immediately before the actual test. This new procedure resulted in a smaller variance then previously, but the repellency index of the controls was reduced (i.e. we obtained a more uniform response, but the overall response level was lower). These data will be subjected to statistical analysis to determine whether or not the conditioning procedure results in a more stable test. All the tests reported in this quarterly report have been performed with the conditioning method. It should be again noted that if this procedure has resulted in reduced biting for the controls, it is also probably correlated with reduced biting for the test compounds. Therefore, the tests reported here maintain their overall validity

It may be profitable at this time to briefly review our underlying objectives in using these methods. Our predominant interest

is to detect small changes which result in significant differences from control values at a high level of confidence. While most other test methods are oriented toward yielding an assured level of protection, that is, toward observing the point at which a predetermined desired level of reduced biting is obtained, our method is oriented toward the screening of possible candidate repellents with maximum sensitivity for testing statistically significant differences from controls. Thus, a certain amount of biting and engorging is permitted before the test compound is rejected. It therefore becomes extremely unlikely to miss possible good repellents due to individual mosquito variation or other uncontrolled or uncontrollable circumstances.

We have also continued with the development of the new electronic "bitometer" described in the last report (ref. 19). The circuitry has been greatly modified so that the instrument is more efficent, and more "fool proof." For instance, if the paw of the mouse should accidentally touch the screen during the test, or the mouse should urinate, essentially causing a short circuit in either case, the instrument automatically shuts itself off and the test becomes invalid. The final stages of testing of this instrument are now in progress, and will be reported upon further in the next quarterly report.

IV. ASSAY OF COMPOUNDS FOR REPELLENCY

The control values upon which the tests of repellency of the following compounds were based are shown in Appendix A. The abbreviated compound names and the treatments listed on the computer program (Appendix A) are defined below.

Computer Listing	Compound Name, Formula and Treatment
AO- and YO- series	Supplied by Dr. R. P. Qaintana of the University of Tennessee. For details of structure, see reference 22.
3-NH2-1-PROPANOL	3-Amino-1-propanol $CH_2(OH)CH_2CH_2(NH_2)$
3-DEA-1-PROPANOL	3-Diethylamino-1-propanol $(C_2H_5)_2NCH_2CH_2CH_2(OH)$
1-DEA-2-PROPANOL	1-Diethylamino-2-propanol $(C_2H_5)_2NCH_2CH(OH)CH_3$
4-DEA-1-BUTANOL	4-Diethylamino-1-butanol $(C_2H_5)_2NCH_2CH_2CH_2CH_2OH$
3-DMA-1-PROPANOL	3-Dimethylamino-1-propanol $(CH_3)_2N(CH_2)_3OH$
1-DMA-2-PROPANOL	1-Dimethylamino-2-propanol $(CH_3)_2NCH_2CH(OH)CH_3$
4-DMA-1-BUTANOL	4-Dimethylamino-1-butanol $(CH_3)_2N(CH_2)_4OH$

Computer Listing	Compound Name, Formula and Treatment
1133TETRAMETHUREA	1,1,3,3-tetramethylurea $(CH_3)_2NCON(CH_3)_2$
DEA ACETONE	Diethylamino acetone $(C_2H_5)_2NCH_2COCH_3$
GABA-ETHYL-ESTER	Gamma-aminobutyrate ethyl ester $(NH_2)CH_2CH_2CH_2COOC_2H_5$
22AMETHOXYETHANOL	2-(2-Aminoethoxy)-ethanol $H_2NCH_2CH_2OCH_2CH_2OH$
3-BUTENE-2-OL	3-Butene-2-ol $CH_3CH(OH)CH{:}CH_2$
4 AMEUTALDDEAHWNB	4-Aminobutyraldehyde diethyl acetal Hydrolyzed in water with hydrochloric acid, neutralized with base
2AM-BENZALDEHYDE	2-Aminobenzaldehyde $o\text{-}H_2N(C_6H_4)CHO$
NNDIETMETCLBENZAM*	N,N-Diethylmetachlorobenzamide $m\text{-}(C_2H_5)_2NCO(C_6H_4)Cl$
4DEAETHOXY BENZAD	4-[β-(Diethylamino)-ethoxy]-benzaldehyde $(C_2H_5)_2NCH_2CH_2OC_6H_4CHO$
NNDIPENYLFORMIDE	N,N-Diphenylformamide $HCON(C_6H_5)_2$

*Submitted for testing by Johnson's Wax Corporation, Racine, Wisconsin.

For all the following assays, the 95% confidence level was accepted as significant.

In the series of compounds assayed for the University of Tennessee, significant repellency was noted for compound A036 at a level of 5.0 mg/sq. in. A035 was repellent at 10.0 mg/sq in., but lost repellency at 5.0 mg/sq. in. A033 was repellent at 30 mg/sq in., but was not significantly repellent at 10 mg/sq in. A032 was significantly repellent at 25 mg/sq in., but not at 10 mg/sq in. Compound A029 was significantly repellent at all levels tested thus far (down to 30 mg/sq in.) and has to be tested further to observe at which point it is no longer repellent. A028 was significantly repellent at 50 mg/sq in., but did not reach the 95% confidence limit at 30 mg/sq in. A016 was significantly repellent at all levels tested (50 and 30 mg/sq in.). A014 was significantly repellent at 50 and 30 mg/sq in., but only reached the 90% confidence limit at 10 mg/sq in. A013 was significantly repellent at all levels tested, down to 5 mg/sq in. Y006 was also significantly repellent at all levels tested, down to 0.1 mg/sq in., but Y007 and Y008 both lost significant repellency at 0.1 mg/sq in.

Those compounds for which we have not seen a loss of repellency for the dilutions have to be tested further with greater dilutions until a definite break point is observed. Those compounds for which repellency is at the 90% level of confidence at the greatest dilution tested will also be further tested to confirm that this dilution is the actual point of repellency loss.

We have observed an occasional reversal of repellency, in that compounds at a higher concentration were not significantly repellent at the 95% level, while the same compound at a lower concentration was significantly repellent. The reason for these reversals cannot be directly accounted for or easily explained. The possibility of such occurrences are implicitly stated in any statistical confidence limit. These occurences may be, though need not necessarily be ascribed to experimental error. Some of these reversals in our testing will be subsequently discussed, and in some cases a more comprehensive evaluation in terms of repeat tests may be indicated.

Appendix A also shows the results of the repellency testing of certain selected compounds in order to continue to test the validity hypothesis previously presented (ref. 9), that analogues of GABA are repellent to mosquitoes.

3-Amino-1-propanol was significantly repellent at 1.0 mg/sq in. but not repellent at 0.1 mg/sq in. On the other hand, the diethyl amino derivative of this compound is repellent at the 0.1 mg/sq in. level. Thus, an electron releasing substituent on the amino group that makes this group more electron rich enhances the repellency of the compound. We have not reached the point of non-repellency at the 0.1 mg/sq in. level with this compound, and it has to be tested at greater dilutions.

If the hydroxyl group is right next to the diethylamino functional group, as in 1-diethylamino-2-propanol, repellency is also retained at the 0.1 mg/sq in. level, but lost with further

dilutions. If the hydroxyl group is removed 2 carbon atoms from the diethylamino functional group, as in 4-diethylamino-1-butanol, repellency is still retained at the 0.1 mg/sq in. level, but misses being significantly repellent at lower levels of application. Thus the distance between functional groups in these tests does not appear to significantly influence repellency.

If methyl groups instead of ethyl groups are the substituents on the amino group, as in 3-dimethylamino-1-propanol, repellency is not evident at the 95% level of confidence even at a concentration of 1.0 mg/sq in. of skin. Here, however, we can see one of the aforementioned reversals. The 3-dimethylamino-1-propanol unexpectedly shows significant repellency at the 0.01 mg/sq in. level, although not at any other level tested. The 1 in 20 statistical chance that such a thing could happen has possibly evidenced itself here. In view of the consistent non-repellency at the other test levels, there appears to be little value in reassessing this compound. These results are consistent with the fact that methyl groups are less electron inducing than ethyl groups.

In 4-dimethylamino-1-butanol the functional groups are separated by two carbon atoms. Here significant repellency is retained even at the .001 mg/sq in. treatment level, and we have not yet reached the non-repellent dilution. The electron withdrawing properties of the hydroxyl group mainly effect carbon atom 2, while carbon atom 3 is free to contribute its electrons to the dimethylamino group to enhance the electron rich properties

of that group. Thus a greater charge differential can be maintained with the butanol derivative than with the propanol derivative. In this respect it is interesting to note that in amino acids, the pK, or basicity of the amino group increases as it is increasingly separated from the carboxyl group. We cannot explain by this line of reasoning however, why 4-diethylamino-1-butanol does not show repellency at least equal to that of the dimethyl derivative.

The compound 1,1,3,3-tetramethyl urea was of interest because here we had the opportunity of testing a symmetrical compound containing substituted amino groups on each side of a carbonyl moiety.

In the tests of this compound, a reversal is again seen. The 0.1 mg/sq in. treatment is significantly repellent, while the 1.0 mg/sq in. treatment is not significantly repellent, nor is any other level of treatment. Again, we have no explanation for these results. In general, however, this compound does not appear to be outstanding as a repellent. In our last report (ref. 19) we suggested the possibility that if the oxygen containing moiety is located in the middle of a molecule, or is surrounded by alkyl groups, the molecules looses its effectiveness as a repellent. This compound appears to offer further evidence of the validity of this hypothesis. The electron-withdrawing properties of the oxygen containing group are neutralized by the inductive properties of the surrounding electron releasing groups.

Further support for this hypothesis is obtained by the results of the assay of diethylaminoacetone. This compound is not repellent at the 1.0 mg/sq in. level or at the 0.1 mg/sq in. level. In this compound, the carbonyl moiety is bonded on both sides to electron inducing groups.

In order to test the effects of the presence of two oxygen atoms in the same molecule with an amino group, we synthesized gamma aminobutyric acid ethyl ester. The synthetic method is described in Appendix B. Although the oxygens in this compound is also surrounded by alkyl moieties, the two oxygens are juxtaposed, and the total electron withdrawing properties of the two oxygens in close proximity should be greater than when only one oxygen is present between alkyl groups. This compound showed significant repellency at 10 mg/sq in. and 1 mg/sq in. We could not test further since we used up all that we had available. Our tests with this compound will continue when more can be obtained. However, it is interesting to note that GABA ethyl ester is significantly repellent at 1.0 mg/sq in. in contrast to the results with 1,1,3,3-tetramethylurea and diethylaminoacetone.

When we tested the repellency of 2-(2-aminoethyoxy)-ethanol, a compound which contains an amino group at one end of the molecule separated by 2 carbons from a mid-chain oxygen, and a hydroxyl group at the other end of the molecule separated by 2 carbons from the same mid-chain oxygen, we found that the compound was no more repellent than any of the omega-amino hydroxy compounds tested here and in the last report (ref. 19). It was significantly

repellent at 1.0 mg/sq in. but not at 0.1 mg/sq in. Apparently, an oxygen atom surrounded by alkyl groups is again shown to contribute little to the electronic polarity of the molecule, and thus has little influence on overall repellency.

In our last report (ref. 18) we tested 3-butene-2-ol and found this compound to be repellent at a concentration of 0.1 mg/sq in. We continued testing this compound during this report period, and found that significant repellency was retained at .01 mg/sq in. but lost at .001 mg/sq in. Thus a compound in which the electron rich structure is in the form of a double bond (pi-bond) exerts as high a level of repellency as a compound in which this structure is in the form of a free or substituted amine. The electronic properties rather than the actual substituents in the molecule therefore appear to be the determining factors in terms of repellent efficacy.

We retested the solution of 4-aminobutyraldehyde diethylacetal which had been hydrolyzed with reflux in the presence of hydrochloric acid and subsequently neutralized with sodium bicarbonate. This is the same solution which we had previously used (ref. 19), and we were interested in assessing the stability of the compound with time. It continued to show significant repellency even at concentrations of .001 mg/sq in. We will continue to test it in further dilutions to observe the point at which significant repellency is lost.

We next studied the effects of the introduction of the benzene ring on the repellency of amino-carbonyl compounds. 2-Aminobenzaldehyde,

a light yellow solid, is sufficiently stable so that it can be obtained commercially as the free aldehyde. This compound was significantly repellent at a concentration of .001 mg/sq in. but lost significant repellency below this level. Therefore, this benzenoid aminoaldehyde is considerably repellent although it is a solid. These results are of great interest when compared to repellency tests of m-aminodiethylbenzamide (ref. 20). This amino analogue of DEET, which is also a solid, was shown to have no repellency even at a concentration of 10 mg/sq in. These results seem to again indicate that neither the precise chemical constitution of a compound nor the distance between the functional groups are as important for repellency as the actual electronic configurations in the molecule. The oxygen containing moiety in the 2-amino benzaldehyde is at an end position, and has no electronic input other than that from the ring, and thus retains its essential electronegativity. On the other hand, the amino analogue of DEET has an oxygen moiety which receives more electrons from the ring because of the inductive effects of amino substitution for the methyl group, as well as from the diethylamide group on its other side.

We also tested another analogue of DEET, namely, N,N-diethyl-m-chloro benzamide. In this compound a chlorine is substituted for the methyl group in the meta position. Chlorine and other halides are known to be deactivating to the benzene ring in terms of electrophilic aromatic substitution. That is, they withdraw electrons from the ring. If our reasoning is correct, then the

essential repellency of the diethylbenzamide group should be retained. We found N,N-diethyl-m-chlorobenzamide to be significantly repellent at all concentrations tested down to .001 mg/sq in. We have not yet reached the concentration limit of repellency for this compound, and it will be tested at greater dilutions.

Continuing along these lines, we tested 4-[β-(Diethylamino)-ethoxy]-benzaldehyde. Here the carbonyl group is free on one side, and an ethoxy group bonds a diethylamine moiety to the aromatic ring. It was surprising to observe that this compound was not significantly repellent at the concentrations tested (1.0 and 0.1 mg/sq in.). It is difficult to interpret these findings directly in terms of our previous lines of reasoning. However, the ethoxy group could conceivably modify the electronic character of either the diethylamine group or the benzene ring so that as a whole the molecule is no longer repellent.

Repellency tests of N,N-diphenylformamide showed that this compound is significantly repellent at 1.0 and 0.1 mg/sq in., and we have not yet reached the repellency limits in terms of concentration in these tests. In this molecule, a free aldehyde is bonded directly to a diphenylamino group. The relationships of an electron rich moiety (diphenylamine) and an electron poor moiety (aldehyde) are retained in the molecule, and repellency is evidenced.

It will be noted in the tables in Appendix A that a number of compounds have one, two, or three asterisks. One asterisk denotes

that the compound is significantly repellent when the control values are multiplied by 0.9, two asterisks denote that it is repellent when the control values are multiplied by 0.75, and three asterisks denote repellency when controls are multiplied by 0.5. In this way, it is possible to judge how much biting is actually reduced from control values in compounds which are shown to be significantly repellent. In these tests, in order for a compound to achieve the 95% confidence level, there must be a reduction of biting by at least 50% of that of controls. If the control values are then further reduced by the above fractions, we can judge by how much less thatn 50% of the controls the tests compounds have reduced biting.

V. SUMMARY

Support has been obtained for the GABA-CO_2 hypothesis from a theoretical consideration in physiochemical terms of the formation and destruction of carbamino compounds.

A modification of the GABA hypothesis has been proposed on the basis of the effects of glutamate in certain crustacean and insect nerve-muscle preparations.

We have found thus far in our repellent testing program that as a general class, amino-aldehydes are highly repellent to mosquitoes. Straight-chain amino alcohols are also repellent, but not as much as amino aldehydes. We have found that 2-aminobenzaldehyde, an aromatic compound, is also highly repellent to mosquitoes. Its

repellency is about the same order of magnitude as the straight-chain amino aldehydes. It is chemically more stable than the straight-chain amino aldehydes. We have not yet tested other types of benzenoid aldehyde-amines, but will do so in future work.

We have found that the amino group in a straight-chain compound may be located either in the middle portion of the molecule, or at the end, but that the oxygen-containing moiety is most effective only if located at the end of the molecule. Therefore, amino-acetones are not as effective as amino aldehydes or amino hydroxyls when the OH group is at the end of the carbon chain. In general, alkyl substituent groups on the amine moiety enhance repellency, while such groups on the oxygen-containing moiety decrease repellency. We have attempted to relate the repellency of these compounds to their electronic configurations rather than to specific functional groups, and in turn relate these electronic configurations to that of the GABA molecule. In most cases these correlations could successfully be shown, and it is proposed that neuro-inhibition is the fundamental basis for the repellency of these compounds.

VI. FUTURE WORK

We will complete the development of the new "bitometer" which we have described. We are building 3 of these new instruments. When they are available, our capability and efficiency in testing compounds for topical as well as systemic repellent potency will be

enhanced. We will continue along the general lines of our past research. We will continue our investigations of the GABA hypothesis to determine its validity and it consequences in terms of developing an orally effective insect repellent.

VII. PERSONNEL AND RECORDS

The author is grateful to Mr. Clarence Boyle for technical assistance in this work, and to Mr. Merl L. Kardatzke for the development of the statistical methods used in analyzing our results. Mr. Karl Roseman performed the synthesis of the gamma-aminobutyrate ethyl ester.

All data are recorded in Logbooks C17189, C17259, C17495, and C17599. The computer output sheets and electronic chart recordings also form part of our permanent records.

Respectfully submitted,

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APPENDIX A

CONTROL VALUES

CONCENTRATION OF ACTIVE (PERCENT)	MOSQUITOES KILLED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)	Day of Test	Test Number
[illegible]	5.77	63.00	68.77		22	1
[illegible]	5.77	45.72	51.49		22	2
[illegible]	3.85	22.66	26.51		22	3
[illegible]	47.17	83.50	130.67		22	4
[illegible]	18.00	61.76	79.76		23	5
[illegible]	7.69	49.20	56.89		23	6
[illegible]	8.16	43.56	51.72		23	7
[illegible]	8.01	57.58	65.59		23	8
[illegible]	11.76	52.98	64.75		24	9
[illegible]	5.88	44.76	50.64		24	10
[illegible]	12.24	42.38	54.62		24	11
[illegible]	8.43	65.30	73.72		25	12
[illegible]	3.77	53.39	57.16		25	13
[illegible]	52.06	95.84	147.93		25	14
[illegible]	52.27	98.94	151.21		26	15
[illegible]	17.65	67.48	85.13		26	16
[illegible]	62.02	94.86	156.86		27	17
[illegible]	31.34	91.54	122.79		27	18
[illegible]	28.26	95.71	123.97		28	19
[illegible]	52.50	93.09	145.59		28	20
[illegible]	36.00	63.43	99.43		29	21
[illegible]	9.09	52.54	61.63		29	22
[illegible]	10.78	56.13	66.34		30	23
[illegible]	50.24	68.76	118.99		30	24
[illegible]	19.61	67.99	87.60		31	25
[illegible]	11.76	62.36	74.15		31	26
[illegible]	15.38	91.99	107.37		32	27
[illegible]	6.06	55.05	61.05		32	28
[illegible]	7.84	52.81	60.65		33	29
[illegible]	61.11	92.40	153.52		33	30
[illegible]	23.53	97.13	120.66		34	31
[illegible]	48.00	90.35	132.35		34	32
[illegible]	6.25	38.18	44.56		34	33
[illegible]	20.75	73.80	94.56		34	34
[illegible]	14.95	63.12	78.07		35	35
[illegible]	30.00	59.66	89.66		35	36
[illegible]	10.20	53.78	63.98		36	37
[illegible]	22.01	66.08	88.08		36	38
[illegible]	36.73	84.75	121.48		37	39
[illegible]	24.59	69.11	93.60		37	40
[illegible]	22.45	95.98	118.43		37	41
[illegible]	16.47	78.82	95.29		37	42
[illegible]	16.96	92.73	109.71		38	43
[illegible]	10.00	74.15	84.15		38	44

CONTROL VALUES (cont.)

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Day of Test	Test Number
	[illegible]	7.27	65.56	72.83		39	45
	[illegible]	6.61	77.18	83.80		39	46
	[illegible]	14.82	67.01	81.83		40	47
	[illegible]	28.36	85.06	113.38		40	48
	[illegible]	44.15	98.31	144.46		41	49
	[illegible]	37.04	97.45	134.48		42	50
	[illegible]	46.00	95.48	141.48		42	51
	[illegible]	5.66	39.05	44.71		43	52
	[illegible]	65.76	98.40	163.76		44	53
	[illegible]	70.91	87.01	157.92		44	54
	[illegible]	26.46	85.30	111.71		45	55
	[illegible]	34.01	68.82	102.82		45	56
	[illegible]	47.31	97.07	133.36		45	57
	[illegible]	19.01	66.46	76.46		46	58
	[illegible]	52.65	64.65	117.30		46	59
	[illegible]	54.96	68.95	155.81		47	60
	[illegible]	68.67	74.76	142.53		47	61
	[illegible]	40.00	85.14	125.14		47	62
	[illegible]	32.45	96.27	128.92		47	63
	[illegible]	29.17	78.13	107.30		47	64
	[illegible]	33.3[illegible]	78.55	111.[illegible]		47	65
	[illegible]	39.33	86.78	126.11		48	66
	[illegible]	27.45	84.09	111.54		48	67
	[illegible]	16.67	48.73	65.40		49	68
	[illegible]	18.[illegible]	74.74	93.74		49	69
	[illegible]	38.29	78.94	115.24		49	70
	[illegible]	14.[illegible]	58.90	72.90		49	71
	[illegible]	30.00	94.17	124.17		49	72
	[illegible]	46.15	84.19	130.34		50	73
	[illegible]	18.[illegible]	73.20	92.19		50	74
	[illegible]	3.[illegible]	14.91	18.51		50	75
	[illegible]	4.00	22.85	26.85		50	76
	[illegible]	3.[illegible]	48.56	52.40		51	77
	[illegible]	8.06	61.56	69.66		51	78
	[illegible]	21.57	57.84	79.41		52	79
	[illegible]	8.[illegible]	51.81	59.81		52	80
	[illegible]	7.[illegible]	33.30	40.84		52	81
	[illegible]	17.91	60.09	77.40		53	82
	[illegible]	28.[illegible]	65.27	94.20		53	83
	[illegible]	4.78	38.58	42.84		54	84
	[illegible]	5.77	38.67	44.43		54	85
	[illegible]	20.41	77.38	97.79		55	86
	[illegible]	26.[illegible]	63.45	119.87		55	87
	[illegible]	14.81	75.80	90.61		56	88

CONTROL VALUES (cont.)

CONTROL SET	CONCENTRATION RELATIVE TO CONTROL (PCT)	RESISTANCES REJECTED (PCT)	TIME DISPLACED (PCT)	PERPLEXITY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
	[illegible]	25.85	77.45	106.70			56	89
	[illegible]	17.65	79.83	97.48			57	90
	[illegible]	14.33	63.31	79.63			57	91
	[illegible]	7.31	60.99	68.30			57	92
	[illegible]	12.71	50.66	63.66			58	93
	[illegible]	33.19	96.46	129.64			58	94
	[illegible]	19.76	78.17	98.93			59	95
	[illegible]	24.63	89.38	113.80			59	96
	[illegible]	12.89	50.11	63.07			60	97
	[illegible]	21.71	49.72	77.50			60	98
	[illegible]	14.00	71.19	85.19			61	99
	[illegible]	22.64	83.31	105.98			61	100
	[illegible]	13.46	59.72	73.19			62	101
	[illegible]	7.69	37.62	45.31			62	102
	[illegible]	7.97	64.88	72.15			63	103
	[illegible]	19.61	55.37	74.98			63	104
	[illegible]	25.45	81.82	107.28			64	105
	[illegible]	38.19	69.09	118.28			64	106
	[illegible]	24.44	61.16	105.60			65	107
	[illegible]	22.64	82.49	105.13			65	108
CONTROL	[illegible]	21.00	70.34	94.34	N.S.	-0.0000=CONTRAST		
		15.95	14.74	33.21		3.715=STANDARD ERROR		

ANALYSIS OF VARIANCE OF CONTROLS

EFFECT	S.S.	D.F.	M.S.	F
[illegible]	71231.035	43.	1634.561	2.193
ERROR	43716.[illegible]	24.	745.569	
TOTAL	114607.[illegible]	107.	1102.526	

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES

COMPOUND NAME	CONCENTRATION OF DOSE (MG/SQ.INCH)	MOSQUITOES PASSAGES (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
AC34								
	50.000000	0.00	0.00	0.00				
	50.000000	0.00	3.21	3.21			34	1
							34	2
AC34****	50.000000	0.00	1.60	1.60	99.5	96.4296=CONTRAST		
		-0.00	2.27	2.27		23.6469=STANDARD ERROR		
AC35								
	30.000000	6.38	17.60	23.98				
	30.000000	5.77	70.68	76.45			33	1
							33	2
AC36	30.000000	6.08	44.14	50.21	95	56.8694=CONTRAST		
		0.63	37.54	37.10		27.3051=STANDARD ERROR		
AC35								
	30.000000	4.00	19.44	23.44				
	30.000000	10.20	63.00	73.20			33	1
							33	2
AC36	30.000000	7.10	41.22	48.32	95	58.7636=CONTRAST		
		4.30	30.80	35.1		27.3051=STANDARD ERROR		
AC36								
	5.000000	2.14	15.55	17.68				
	5.000000	7.55	49.04	56.59			34	1
							34	2
AC36*	5.000000	4.84	32.30	37.13	97.5	60.8984=CONTRAST		
		3.83	23.68	27.51		23.6469=STANDARD ERROR		
AC35								
	30.000000	0.00	0.00	0.00				
	30.000000	0.00	0.00	0.00			32	1
							32	2
AC35*	30.000000	0.00	0.00	0.00	99.5	84.2143=CONTRAST		
		-0.00	-0.00	-0.00		27.3051=STANDARD ERROR		

REPELLENCY OF CO[illegible] CURES CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND TYPE	CONCENTRATION OF DOSE (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
AC35	10.00000	7.55	48.06	55.61			32	1
	10.00000	0.00	0.00	0.00			32	2
AC35	10.00000	3.77	24.03	27.80	95	56.4097=CONTRAST 27.3051=STANDARD ERROR		
		5.44	33.98	39.32				
AC35	5.00000	14.98	81.08	98.06			34	1
	5.00000	3.32	33.92	37.84			34	2
AC35	5.00000	10.45	57.50	67.95	N.S.	30.0808=CONTRAST 23.6469=STANDARD ERROR		
		3.23	33.35	42.58				
AC32	75.00000	0.00	26.83	26.83			28	1
AC32**	75.00000	0.00	26.83	26.83	99.5	96.1627=CONTRAST 31.5292=STANDARD ERROR		
		-0.00	-0.00	-0.00				
AC32	50.00000	0.00	0.00	0.00			28	1
AC32**	50.00000	0.00	0.00	0.00	99.5	122.9960=CONTRAST 31.5292=STANDARD ERROR		
		-0.00	-0.00	-0.00				
AC32	25.00000	0.00	0.00	0.00			28	1
AC32**	25.00000	0.00	0.00	0.00	99.5	122.9960=CONTRAST 31.5292=STANDARD ERROR		
		-0.00	-0.00	-0.00				

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION ON MOUSE (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
A032								
	10.00000	15.63	68.05	83.74			29	1
	10.00000	1.85	34.56	36.42			34	2
A032	10.00000	3.77	51.31	69.08	N.S.	29.4145=CONTRAST		
		9.78	23.68	33.46		23.9482=STANDARD ERROR		
A033								
	50.00000	0.00	0.00	0.00			31	1
A033*	50.00000	0.00	0.00	0.00	97.5	80.8720=CONTRAST		
		-0.00	-0.00	-0.00		33.4418=STANDARD ERROR		
A033								
	30.00000	0.00	13.66	13.66			31	1
A033	30.00000	0.00	13.66	13.66	95	67.2084=CONTRAST		
		-0.00	-0.00	-0.00		33.4418=STANDARD ERROR		
A033								
	10.00000	2.27	16.58	18.86			30	1
	10.00000	22.45	70.23	92.68			30	2
A033	10.00000	12.36	43.41	55.77	80	36.8972=CONTRAST		
		14.27	37.93	52.20		27.3051=STANDARD ERROR		
A029								
	50.00000	0.00	5.76	5.76			40	1
	50.00000	1.92	13.90	15.82			40	2
A029**	50.00000	0.96	9.83	10.79	99.5	86.8136=CONTRAST		
		1.36	5.75	7.11		27.3051=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION ON MOUSE (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
A029	30.00000	0.00	0.00	0.00			40	1
	30.00000	0.00	3.77	3.77			40	2
A029**	30.00000	0.00	1.88	1.88	99.5	95.7219=CONTRAST		
		-0.00	2.67	2.67		27.3051=STANDARD ERROR		
A028	50.00000	0.00	0.00	0.00			39	1
	50.00000	0.00	0.00	0.00			39	2
A028**	50.00000	0.00	0.00	0.00	99.5	79.8145=CONTRAST		
		-0.00	-0.00	-0.00		27.3051=STANDARD ERROR		
A028	30.00000	3.77	31.61	35.39			39	1
	30.00000	3.44	16.63	20.27			39	2
A028	30.00000	3.70	24.12	83	90	51.9876=CONTRAST		
		0.10	10.60	9		27.3051=STANDARD ERROR		
A016	50.00000	0.00	0.00	0.00			38	1
	50.00000	0.00	0.00	0.00			38	2
A016**	50.00000	0.00	0.00	0.00	99.5	96.9294=CONTRAST		
		-0.00	-0.00	-0.00		27.3051=STANDARD ERROR		
A016	30.00000	2.00	8.18	10.18			38	1
	30.00000	6.00	47.99	53.99			38	2
A016*	30.00000	4.00	28.08	32.08	97.5	64.8471=CONTRAST		
		2.83	28.15	30.98		27.3051=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION ON MOUSE (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
A014	50.00000	1.92	9.89	11.82			41	1
	50.00000	9.26	42.32	51.58			37	2
A014**	50.00000	5.59	26.11	31.70	99.5	85.2445=CONTRAST 23.9482=STANDARD ERROR		
		5.19	22.93	28.12				
A014	30.00000	4.04	34.23	38.31			37	1
	30.00000	0.00	0.00	0.00			37	2
A014**	30.00000	2.04	17.12	19 16	99.5	88.0442=CONTRAST 23.6469=STANDARD ERROR		
		2.89	24.21	27.09				
A014	10.00000	24.00	91.34	115.34			37	1
	10.00000	2.08	8.37	10.46			37	2
A014	10.00000	13.04	49.86	62.90	90	44.3019=CONTRAST 23.6469=STANDARD ERROR		
		15.50	58.67	74.16				
A013	30.00000	0.00	0.00	0.00			36	1
	30.00000	0.00	0.00	0.00			36	2
A013**	30.00000	0.00	0.00	0.00	99	76.0296=CONTRAST 27.3051=STANDARD ERROR		
		-0.00	-0.00	-0.00				
A013	10.00000	0.00	0.00	0.00			35	1
	10.00000	3.70	19.43	23.13			35	2
	10.00000	16.33	68.38	84.71			42	3
	10.00000	24.49	63.54	88.03			42	4
A013*	10.00000	11.13	37.84	48.97	99.5	61.9540=CONTRAST 19.3076=STANDARD ERROR		
		11.32	33.49	44.23				

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

Compound	Concentration of Vapor (mg/meter cubed)	Mosquitoes Responded (Pct)	Time Displaced (Pct)	Repellency Index	Confidence Level (Pct)		Day of Test	Test Number
A013	[illegible]	[illegible]	27.44	31.36			35	1
	[illegible]	[illegible]	6.53	6.53			35	2
	[illegible]	[illegible]	28.49	32.49			37	3
	[illegible]	[illegible]	65.48	80.06			42	4
	[illegible]	14.33	78.17	94.50			42	5
A013**	[illegible]	[illegible]	41.22	48.99	99.5	62.6369=CONTRAST		
		[illegible]	29.62	36.82		16.3179=STANDARD ERROR		
Y006	[illegible]	[illegible]	34.00	35.96			44	1
	[illegible]	0.0	0.00	0.00			44	2
Y006***	[illegible]	[illegible]	17.00	17.98	99.5	142.8713=CONTRAST		
		[illegible]	24.04	25.43		27.3051=STANDARD ERROR		
Y006	[illegible]	20.0	50.86	70.86			44	1
	[illegible]	26.53	80.10	106.63			44	2
Y006*	[illegible]	23.27	65.48	88.75	97.5	72.1042=CONTRAST		
		4.62	20.67	25.29		27.3051=STANDARD ERROR		
Y007	[illegible]	0.0	0.00	0.00			45	1
Y007**	[illegible]	[illegible]	0.00	0.00	99.5	118.6375=CONTRAST		
		-0.00	-0.00	-0.00		31.5292=STANDARD ERROR		
Y007	[illegible]	[illegible]	2.77	2.77			45	1
	[illegible]	[illegible]	18.85	20.85			45	2
Y007**	[illegible]	[illegible]	10.81	11.81	99.5	106.8312=CONTRAST		
		1.41	11.37	12.79		24.9260=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION ON MOUSE (MG/50.8INCH)	MOSQUITOES ENGORGED (PCT)	TIME % DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
YOO7	0.100000	5.00	65.59	79.58			45	1
	0.100000	40.50	97.65	140.25			45	2
YOO7	0.100000	25.30 24.46	81.61 22.68	106.91 47.14	N.S.	11.7263=CONTRAST 24.9260=STANDARD ERROR		
YOO3	1.000000	2.00	21.89	23.93			46	1
	1.000000	4.00	13.71	17.71			46	2
YOO8 *	1.000000	3.00 1.41	17.80 5.78	20.82 4.40	99	76.0729=CONTRAST 27.3051=STANDARD ERROR		
YOO8	0.100000	3.00	36.43	40.87			46	1
	0.100000	9.00	69.90	78.99			46	2
YOOA	0.100000	6.00 3.24	53.16 23.67	59.93 26.95	80	36.9583=CONTRAST 27.3051=STANDARD ERROR		
3-NH2-1-PROPANOL	1.000000	0.00	0.00	0.00			23	1
	1.000000	0.00	0.00	0.00			23	2
3-NH2-1-PROPANOL **	1.000000	0.00 -0.00	0.00 -0.00	0.00 -0.00	99	63.4886=CONTRAST 23.6469=STANDARD ERROR		
3-NH2-1-PROPANOL	0.100000	5.88	20.44	25.92			23	1
	0.100000	1.96	16.23	18.19			23	2
3-NH2-1-PROPANOL	0.100000	3.92 2.77	18.34 2.98	22.26 5.75	90	41.2290=CONTRAST 23.6469=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION ON MOUSE (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
3-DEA-1-PROPANOL	1.00000	0.00	4.30	4.30			27	1
	1.00000	0.00	0.00	0.00			27	2
3-DEA-1-PROPANOL ***	1.00000	0.00	2.15	2.15	99.5	137.6743=CONTRAST		
		-0.00	3.04	3.04		27.3051=STANDARD ERROR		
3-DEA-1-PROPANOL	0.10000	2.13	36.48	34.60			27	1
	0.10000	11.11	58.46	69.57			27	2
3-DEA-1-PROPANOL *	0.10000	5.62	47.47	54.09	99.5	85.7395=CONTRAST		
		6.95	15.54	21.40		27.3051=STANDARD ERROR		
1-DEA-2-PROPANOL	1.00000	0.00	0.00	0.00			53	1
	1.00000	0.00	0.00	0.00			53	2
1-DEA-2-PROPANOL **	1.00000	0.00	0.00	0.00	99.5	86.7964=CONTRAST		
		-0.00	-0.00	-0.00		27.3051=STANDARD ERROR		
1-DEA-2-PROPANOL	0.10000	2.00	21.67	23.67			53	1
	0.10000	5.48	31.37	37.26			53	2
1-DEA-2-PROPANOL	0.10000	3.94	26.52	30.46	95	56.3332=CONTRAST		
		2.75	6.86	9.61		27.3051=STANDARD ERROR		
1-DEA-2-PROPANOL	0.01000	4.44	31.36	35.80			57	1
	0.01000	8.16	46.12	54.28			57	2
1-DEA-2-PROPANOL	0.01000	6.30	38.74	45.04	80	36.7952=CONTRAST		
		2.63	10.44	13.07		24.9260=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION ON MOUSE (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
1-DEA-2-PROPANOL	0.00100	0.00	0.00	0.00			57	1
	0.00100	8.33	48.10	56.43			57	2
1-DEA-2-PROPANOL	0.00100	4.17 5.89	24.05 34.01	28.22 39.90	95	53.6194=CONTRAST 24.9260=STANDARD ERROR		
4-DEA-1-BUTANOL	1.00000	0.00	0.00	0.00			23	1
	1.00000	0.00	0.00	0.00			23	2
4-DEA-1-BUTANOL**	1.00000	0.00 -0.00	0.00 -0.00	0.00 -0.00	99	63.4886=CONTRAST 23.6469=STANDARD ERROR		
4-DEA-1-BUTANOL	0.10000	0.00	0.00	0.00			23	1
	0.10000	0.00	0.00	0.00			23	2
4-DEA-1-BUTANOL**	0.10000	0.00 -0.00	0.00 -0.00	0.00 -0.00	99	63.4886=CONTRAST 23.6469=STANDARD ERROR		
4-DEA-1-BUTANOL	0.01000	0.00	0.00	0.00			24	1
	0.01000	0.00	13.90	13.90			24	2
4-DEA-1-BUTANOL	0.01000	0.00 -0.00	6.95 9.83	6.95 9.83	90	49.7183=CONTRAST 24.9260=STANDARD ERROR		
4-DEA-1-BUTANOL	0.00100	0.00	0.00	0.00			24	1
	0.00100	4.08	24.88	28.96			24	2
4-DEA-1-BUTANOL	0.00100	2.04 2.89	12.44 17.59	14.48 20.48	90	42.1905=CONTRAST 24.9260=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION DOSE (MG/SQ.INCH)	MOSQUITOES ENGAGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
[illegible]								
	[illegible]	11.77	59.26	71.03			56	1
	[illegible]	11.11	53.44	64.55			56	2
[illegible]	[illegible]	11.44	56.35	67.79	N.S.	30.8689=CONTRAST		
		0.46	4.12	4.56		27.3051=STANDARD ERROR		
[illegible]								
	[illegible]	14.81	78.61	93.43			56	1
	[illegible]	31.48	76.50	107.98			56	2
[illegible]	[illegible]	23.15	77.56	100.71	N.S.	-2.0514=CONTRAST		
		11.77	1.49	10.29		27.3051=STANDARD ERROR		
[illegible]								
	[illegible]	0.00	0.00	0.00			60	1
	[illegible]	0.00	6.67	6.67			60	2
[illegible]*	[illegible]	0.00	3.33	3.33	97.5	66.9542=CONTRAST		
		-2.00	4.71	4.71		27.3051=STANDARD ERROR		
[illegible]								
	[illegible]	1.92	23.61	25.53			60	1
	[illegible]	3.85	41.82	45.67			60	2
[illegible]	[illegible]	2.88	32.72	35.60	N.S.	34.6857=CONTRAST		
		1.36	12.58	14.24		27.3051=STANDARD ERROR		
[illegible]								
	[illegible]	7.55	65.16	72.71			58	1
	[illegible]	5.77	30.00	35.77			58	2
[illegible]	[illegible]	6.66	47.58	54.24	60	41.9109=CONTRAST		
		1.26	24.87	26.12		27.3051=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION ON MOUSE (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	PEPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
1-DMA-2-P,OPFIOL	[illegible]	7.85	31.66	35.50			58	1
	[illegible]	12.50	59.95	72.45			58	2
1-DMA-2-PROPFIOL	[illegible]	8.17	45.80	53.97	80	42.1770=CONTRAST		
		6.12	20.00	26.12		27.3051=STANDARD ERROR		
1-DMA-2-PROPFIOL	[illegible]	4.25	38.56	42.81			59	1
	[illegible]	2.96	24.64	26.60			59	2
1-DMA-2-PROPFIOL*	[illegible]	3.11	31.60	34.71	97.5	71.7075=CONTRAST		
		1.62	9.84	11.46		27.3051=STANDARD ERROR		
1-DMA-2-PROPFIOL	[illegible]	5.53	77.73	83.29			59	1
	[illegible]	12.24	69.16	82.37			59	2
1-DMA-2-PROPFIOL	[illegible]	8.38	73.45	82.83	N.S.	23.5868=CONTRAST		
		5.41	6.38	0.65		27.3051=STANDARD ERROR		
4-DMA-1-BUTANOL	[illegible]	0.00	0.00	0.00			25	1
	[illegible]	0.00	0.00	0.00			25	2
4-DMA-1-BUTANOL **	[illegible]	0.00	0.00	0.00	99.5	92.9379=CONTRAST		
		-0.00	-0.00	-0.00		24.9260=STANDARD ERROR		
4-DMA-1-BUTANOL	[illegible]	0.00	0.00	0.00			25	1
	[illegible]	0.00	0.00	0.00			25	2
4-DMA-1-BUTANOL **	[illegible]	0.00	0.00	0.00	99.5	92.9379=CONTRAST		
		-0.00	-0.00	-0.00		24.9260=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION OF DOSE (MG/SQ.FT.)	MOSQUITOES REPELLED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
[illegible]	[illegible]	[illegible]	0.00	0.00			25	1
	[illegible]	[illegible]	0.00	0.00			25	2
[illegible]	[illegible]	0.0	0.00	0.00	99.5	92.9379=CONTRAST		
		-0.0	-0.00	-0.00		24.9260=STANDARD ERROR		
[illegible]								
	[illegible]	[illegible]	0.00	0.00			25	1
	[illegible]	[illegible]	29.47	35.24			25	2
[illegible]	[illegible]	2.94	14.74	17.62	99.5	75.3165=CONTRAST		
		4.09	20.64	24.92		24.9260=STANDARD ERROR		
1193TETRAMETHRIN								
	[illegible]	4.04	47.07	51.15			48	1
	[illegible]	18.01	55.72	73.72			48	2
1193TETRAMETHRIN	[illegible]	11.64	51.40	62.44	90	53.3874=CONTRAST		
		5.84	6.12	15.96		27.3051=STANDARD ERROR		
1193TETRAMETHRIN								
	[illegible]	[illegible]	0.00	0.00			48	1
	[illegible]	4.04	27.40	31.40			48	2
	[illegible]	14.29	57.57	71.85			49	3
1193TETRAMETHRIN *	[illegible]	6.12	28.32	34.45	99.5	64.5142=CONTRAST		
		7.36	28.80	36.02		20.1662=STANDARD ERROR		
1193TETRAMETHRIN								
	[illegible]	1.92	17.91	19.83			62	1
	[illegible]	[illegible]	2.49	2.49			62	2
1193TETRAMETHRIN	[illegible]	9.04	10.20	11.16	90	48.0902=CONTRAST		
		1.96	10.90	12.26		27.3051=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND TESTED	CONCENTRATION OF DOSE (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
[illegible]	[illegible]	[illegible]	4.25	4.25			62	1
	[illegible]	[illegible]	9.25	9.25			62	2
[illegible]	[illegible]	[illegible]	6.75	6.75	90	52.4949=CONTRAST		
		-20.00	3.53	3.53		27.3051=STANDARD ERROR		
[illegible]								
	[illegible]	[illegible]	66.93	81.21			49	1
	[illegible]	[illegible]	45.91	55.91			49	2
[illegible]	[illegible]	[illegible]	56.42	68.56	N.S.	25.1254=CONTRAST		
		[illegible]	14.86	17.86		22.8451=STANDARD ERROR		
[illegible]								
	[illegible]	[illegible]	69.94	82.44			49	1
	[illegible]	[illegible]	44.46	49.15			49	2
[illegible]	[illegible]	[illegible]	57.21	65.79	N.S.	27.9031=CONTRAST		
		[illegible]	18.01	23.56		22.8451=STANDARD ERROR		
[illegible]								
	[illegible]	[illegible]	67.51	78.62			47	1
	[illegible]	[illegible]	22.95	27.04			47	2
[illegible]	[illegible]	[illegible]	45.23	52.83	99.5	75.6004=CONTRAST		
		[illegible]	31.51	36.46		22.2945=STANDARD ERROR		
[illegible]								
	[illegible]	[illegible]	77.31	92.31			47	1
	[illegible]	[illegible]	36.19	40.03			47	2
[illegible]	[illegible]	[illegible]	54.25	66.17	99	62.2614=CONTRAST		
		[illegible]	25.54	36.96		22.2945=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION OF RELEASE (MG/SQ.INCH)	MOSQUITOES FAVORED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
2[illegible]OL								
	[illegible]	0.00	0.00	0.00			65	1
	[illegible]	16.96	44.74	61.72			65	2
2[illegible]OL*	[illegible]	8.48	22.37	30.86	99	74.5049=CONTRAST		
		7.03	31.63	43.64		27.3051=STANDARD ERROR		
2[illegible]OL								
	[illegible]	[illegible]	62.20	82.26			65	1
	[illegible]	17.32	37.40	54.71			65	2
2[illegible]OL	[illegible]	18.65	49.80	68.46	80	36.9072=CONTRAST		
		1.91	17.54	19.44		27.3051=STANDARD ERROR		
3-BUTENE-2-OL								
	[illegible]	5.60	24.72	30.36			55	1
	[illegible]	10.01	55.67	65.67			55	2
3-BUTENE-2-OL	0.01000	7.83	40.20	48.03	95	60.8017=CONTRAST		
		3.07	21.89	24.95		27.3051=STANDARD ERROR		
3-BUTENE-2-OL								
	0.00100	20.00	76.97	96.97			55	1
	0.00100	6.38	35.02	41.41			55	2
3-BUTENE-2-OL	[illegible]	13.19	56.00	69.19	80	39.6433=CONTRAST		
		9.63	29.66	39.29		27.3051=STANDARD ERROR		
4-METHYL-3-PENTEN-2-ONE								
	1.00000	0.00	0.00	0.00			24	1
	1.00000	0.00	0.00	0.00			24	2
4-METHYL-3-PENTEN-2-ONE*	1.00000	0.00	0.00	0.00	95	56.6640=CONTRAST		
		-0.00	-0.00	-0.00		24.9260=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND	CONCENTRATION IN DOSE (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
[illegible]								
	0.10000	0.00	0.00	0.00			24	1
	0.10000	0.00	0.00	0.00			24	2
	0.10000	0.00	26.12	26.12			50	3
	0.10000	8.00	33.70	41.70			50	4
[illegible]	0.10000	2.00	14.95	16.95	97.5	43.9818=CONTRAST		
		4.00	17.54	20.59		17.1593=STANDARD ERROR		
[illegible]								
	0.01000	14.00	61.16	75.16			26	1
	0.01000	1.96	5.96	7.92			26	2
	0.01000	5.56	35.75	41.30			50	3
	0.01000	0.00	15.97	15.97			50	4
[illegible]	0.01000	5.38	29.71	35.09	99.5	54.4627=CONTRAST		
		6.19	24.35	30.27		17.8754=STANDARD ERROR		
[illegible]								
	0.00100	6.52	53.35	59.87			26	1
	0.00100	4.00	25.00	29.00			26	2
[illegible]	0.00100	5.26	39.18	44.44	99	73.7339=CONTRAST		
		1.75	20.05	21.83		27.3051=STANDARD ERROR		
2-M-[illegible]								
	1.00000	0.00	0.00	0.00			50	1
	1.00000	0.00	0.00	0.00			50	2
	1.00000	0.00	0.00	0.00			52	3
	1.00000	0.00	0.00	0.00			52	4
2-M-[illegible]	1.00000	0.00	0.00	0.00	99.5	63.4157=CONTRAST		
		-0.00	-0.00	-0.00		17.1553=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION ON WOOL (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
PAM-PENZALDEHYDE								
	0.10000	0.00	0.00	0.00			50	1
	0.10000	0.00	0.00	0.00			50	2
	0.10000	0.00	0.00	0.00			52	3
	0.10000	0.00	0.00	0.00			52	4
PAM-PENZALDEHYDE**	0.10000	0.00	0.00	0.00	99.5	63.4157=CONTRAST		
		-0.00	-0.00	-0.00		17.1543=STANDARD ERROR		
PAM-PENZALDEHYDE								
	0.01000	0.00	0.00	0.00			51	1
	0.01000	0.00	0.00	0.00			51	2
	0.01000	0.00	2.40	2.40			52	3
	0.01000	0.00	0.00	0.00			52	4
PAM-PENZALDEHYDE**	0.01000	0.00	0.60	0.60	99.5	59.8258=CONTRAST		
		-0.00	1.20	1.20		18.4091=STANDARD ERROR		
PAM-PENZALDEHYDE								
	0.00100	0.00	0.00	0.00			51	1
	0.00100	3.77	50.00	53.77			51	2
	0.00100	3.85	51.25	55.10			52	3
	0.00100	1.89	19.36	21.25			52	4
	0.00100	2.04	17.95	19.99			61	5
	0.00100	0.00	0.00	0.00			61	6
PAM-PENZALDEHYDE*	0.00100	1.92	23.09	25.02	99.5	45.6061=CONTRAST		
		1.70	22.91	24.59		15.2640=STANDARD ERROR		
PAM-PENZALDEHYDE								
	0.00010	4.26	13.41	17.67			54	1
	0.00010	6.25	16.96	23.21			54	2
	0.00010	16.26	78.78	95.06			61	3
	0.00010	0.00	0.00	0.00			61	4
PAM-PENZALDEHYDE	0.00010	6.70	27.29	33.99	90	35.6181=CONTRAST		
		6.90	35.10	41.90		19.3076=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION ON FOLSE (MG/SQ.INCH)	MOSQUITOES ENGORGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
PAM-BENZALDEHYDE								
	0.00001	4.00	17.79	23.79			54	1
	0.00001	4.17	24.94	29.11			54	2
PAM-BENZALDEHYDE	0.00001	5.08	21.37	26.45	N.S.	17.1847=CONTRAST		
		1.30	5.06	3.76		27.3051=STANDARD ERROR		
PARIETHYLBENZAM								
	1.00000	0.00	0.00	0.00			63	1
	1.00000	0.00	0.00	0.00			63	2
PARIETHYLBENZAM**	1.00000	0.00	0.00	0.00	99	73.5639=CONTRAST		
		-0.00	-0.00	-0.00		27.3051=STANDARD ERROR		
PARIETHYLBENZAM								
	0.10000	0.00	0.00	0.00			63	1
	0.10000	0.00	0.00	0.00			63	2
PARIETHYLBENZAM**	0.10000	0.00	0.00	0.00	99	73.5639=CONTRAST		
		-0.00	-0.00	-0.00		27.3051=STANDARD ERROR		
PARIETHYLBENZAM								
	0.01000	4.17	15.76	19.92			64	1
	0.01000	1.96	4.92	6.88			64	2
PARIETHYLBENZAM**	0.01000	3.06	10.34	13.40	99.5	99.3773=CONTRAST		
		1.56	7.67	9.23		27.3051=STANDARD ERROR		
PARIETHYLBENZAM								
	0.00100	1.96	13.20	15.17			64	1
	0.00100	6.38	30.63	37.02			64	2
PARIETHYLBENZAM**	0.00100	4.17	21.92	26.09	99.5	86.6875=CONTRAST		
		3.13	12.32	15.45		27.3051=STANDARD ERROR		

REPELLENCY OF COMPOUNDS CONTRASTED WITH CONTROL VALUES (Cont.)

COMPOUND NAME	CONCENTRATION OF MIST (GM/CU.INCH)	MOSQUITOES ENGAGED (PCT)	TIME DISPLACED (PCT)	REPELLENCY INDEX	CONFIDENCE LEVEL (PCT)		Day of Test	Test Number
[illegible]								
	1.00000	31.91	68.13	100.04			47	1
	1.00000	20.41	64.04	84.44			47	2
[illegible]	1.00000	24.16	66.19	92.04	80	36.1879=CONTRAST		
		3.14	2.39	11.03		22.2945=STANDARD ERROR		
[illegible]								
	0.10000	32.90	78.15	110.15			47	1
	0.10000	18.00	46.94	64.94			47	2
[illegible]	0.10000	25.90	62.55	87.55	90	40.8609=CONTRAST		
		9.90	22.07	31.97		22.2945=STANDARD ERROR		
[illegible]								
	1.00000	6.72	27.62	33.75			49	1
	1.00000	7.69	42.49	50.19			49	2
[illegible]	1.00000	6.91	35.06	41.97	95	51.7206=CONTRAST		
		1.11	10.51	11.62		22.8451=STANDARD ERROR		
[illegible]								
	0.10000	3.85	50.36	54.21			49	1
	0.10000	3.85	24.06	27.90			49	2
[illegible]	0.10000	3.85	37.21	41.05	97.5	82.6327=CONTRAST		
		-3.90	18.60	18.60		22.8451=STANDARD ERROR		

*Retains significance at the 95% level at 0.9 of control values.

**Retains significance at the 95% level at 0.75 of control values.

***Retains significance at the 95% level at 0.5 of control values.

APPENDIX B

SYNTHESIS OF THE ETHYL ESTER OF 4-AMINOBUTYRATE

Ethyl-4-aminobutyrate was prepared by the Fisher esterification procedure employing absolute ethanol, hydrogen chloride gas and 4-aminobutyric acid. The ethanol was saturated with hydrogen chloride, and acid added and the solution was allowed to stand at room temperature for one week. The solvent was removed, residue dissolved in a small amount of water and neutralized with sodium carbonate. The basicified solution was continuously extracted with ether for 21 hours, followed by drying of the ethereal extract and removal of the ether at atmospheric pressure. Low vacuum distillation of the residue yielded a colorless distillate, B.P. 24mm Hg = 94.5-95.0°; n_D^{20} 1.4332. Proton Magnetic resonance (P.M.R.) spectral data show this to be the ethyl 4-aminobutyrate (Lit.* B.P. 12mm Hg = 75-77°).

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*Abderhalden and Klautsch, H. 81, 304 (Bielstein 4, I, 506).